

SQUIRRELL et al.
Appl. No. 09/763,824
April 15, 2005

AMENDMENTS TO THE DRAWINGS

The attached eight (8) sheets of drawings marked "Replacement Sheet" include changes to Figs. 1 and 3, as required by the Examiner in ¶[9] on page 5 of the Office Action dated December 15, 2004. Also attached is eight (8) sheets of drawings marked "Annotated Marked-up Drawings" which include hand written text showing the changes in the originally-filed drawings.

Attachment: 8 Replacement Sheets
8 Annotated Sheets Showing Changes

REMARKS

Reconsideration is requested.

Claims 1-30 have been canceled, without prejudice. Claims 31-66 have been added. Claims 31-59 are similar to claims 1-26 and 28-30. Claims 36, 39-50 and 56 are similar to withdrawn claims 6, 9-20 and 26.

A Rule 181 Petition is attached requesting the Director to invoke his supervisory authority for reconsideration and withdrawal of the restriction requirement.

Consideration and a Decision on the attached Petition, prior to issuance of a further Office Action are requested as the Director's Decision on the Petition may change the scope of examination.

The drawings have been amended to include new figure numbers. Acceptance of the drawings in the Examiner's next Action is requested.

The Examiner's objection of the Sequence Listing and/or specification based on an alleged lack of compliance of the sequence rules is believed to be unfounded. The Examiner is requested to see the applicants' amendment to the specification dated August 19, 2002. The Examiner is requested to advise the undersigned if anything further is required in this regard.

The specification has been amended to capitalize the trademark terms indicated by the Examiner in paragraph 11 on page 6 of the December 15, 2004, Office Action. A generic description of the terminology has also been included or was originally-contained in the specification.

The specification has been amended to include the new title which is believed to represent the applicant's claimed invention. As a Rule 181 petition has been filed in

relation to the restriction requirement, amending the title as suggested by the Examiner in paragraph 12 on page 7 of the December 15, 2004, Office Action is not believed to be appropriate at the present time.

The specification has been amended to include the title "Brief Description of the Drawings", as required by the Examiner in paragraph 13 on page 7 of the Office Action dated December 15, 2004.

The objection to claims 1, 4-5 and 7-8 is moot in view of the above amendments however 31, 34, 35, 37 and 38 are similar to the objected claims and the Examiner is requested to hold the objection in abeyance with regard to these claims until such time as the issue of the restriction requirement is completely decided.

The Section 112, second paragraph, rejection of claims 1, 2-5, 7-8, 21-25 and 28-30 is moot in view of the above. The claims are submitted to be definite and consideration of the following in this regard is requested.

a) The Examiner is understood to believe that the cancelled claims were indefinite because the sequence of *Photinus pyralis* luciferase, as well as the other wild type luciferases are allegedly not provided in the specification. The Examiner is urged to appreciate however that these sequences are in the public domain, as is clear from the Ye et al. Biochim. Biophys Acta 1339 (1997) 39-52, referred to one page 7 lines 21-23 of the specification. The applicants need not describe in the specification that which is well known to one of ordinary skill in the art. The specification clearly describes that correspondence of particular amino acids should be determined by reference to Ye et al. and the paper sets out the alignment for all the known naturally occurring luciferases. The metes and bounds of the claimed invention will be appreciated by one

of ordinary skill. The sequences and corresponding amino acids positions are taught by the specification.

- b) The claims are believed to provide proper antecedent basis for recited terms and phrases.
- c) While not necessarily believed to be required, the claims refer to identity. The claims are believed to define mutants having an amino acid sequence which is 60% identical to the amino acid sequence of the recited luciferases.
- d) New claims 32 and 33, which are similar to cancelled claims 2 and 3, are believed to be definite.
- e) Claim 57, which is similar to cancelled claim 28, is submitted to be definite.

The claims are submitted to be definite.

The Section 101 rejection of claims 1-5, 7-8 and 21 is moot. Similarly, the Section 101 rejection of claim 28 is moot in view of the above.

The pending claims are believed to define patentable subject matter. Specifically, claim 57, which is similar to now cancelled claim 28, is directed to an improved method which will be recognized by one of ordinary skill in the art. Moreover, claims 31-35, 37-38 and 51 are similar to now cancelled claims 1-5, 7-8 and 21, are not believed to read on a product of nature, which is understood to be the basis for the Examiner's rejection stated in paragraph 16 on page 9 of the Office Action of December 15, 2004. The Examiner is urged to appreciate that the claimed proteins are recombinant proteins that contain at least one mutation and are not believed to read on wild-type proteins. Accordingly, the Examiner's suggested amendments to overcome

the Section 101 rejection are not believed to be required. The Examiner is requested to contact the undersigned however if anything further is required in this regard.

The Section 112, first paragraph, rejection of claims 1-5, 7-8, 21-25 and 28-30 stated in paragraph 18 spanning pages 10-12 of the Office Action dated December 15, 2004, is moot in view of the above. The claims are submitted to be supported by an adequate written description and consideration of the following in this regard is requested.

The Examiner's reference to MPEP § 2163 is appreciated. The applicants submit that the claimed genus has been described by both disclosure of relevant, identifying characteristics, i.e. structure or other physical or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, and by the examples. The applicants believe that one of ordinary skill will understand that the applicants were in possession of the claimed genus at the time the application was filed.

The specification clearly identifies, for example, a specific position within a luciferase protein which is to be mutated in order to achieve the desired result. This has been exemplified using the *Photinus pyralis* luciferase. The description also teaches that the exemplified results are applicable to other members of the claimed genus. One of ordinary skill will appreciate that the specification describes the claimed genus because the ordinarily skilled person will understand that the luciferase proteins form a distinct group of highly conserved proteins. The specification describes the corresponding mutations in the other species sequences by reference to, for example, Ye et al.

For completeness, the applicants urge the Examiner to appreciate that the specification provides more than one example where a *Photinus pyralis* luciferase having a substitution at position 214 and optionally mutations at positions 215, 232 and/or 354 and a nucleic acid encoding therefore, as characterized by the Examiner. Specifically, the specification describes many mutants, and the Examiner is requested to see, for example, the paragraph bridging pages 14-15 and the Examples, in particular example 7.

The applicants submit that one of ordinary skill will appreciate that the claims are adequately described in the specification as filed.

The Section 112, first paragraph, rejection of claims 1-5, 7-8, 21-25 and 28-30 stated in paragraph 19 spanning pages 12-16 of the Office Action dated December 15, 2004, is moot in view of the above. The specification is believed to teach one of ordinary skill in the art how to make and use the claimed invention and consideration of the following in this regard as requested.

Initially, the applicants submit that the Examiner's comments suggesting that one of ordinary skill would require absolute predictability (see, paragraph spanning pages 14-15 of the Office Action dated December 15, 2004) are not an accurate reflection of the approach which would be taken by one of ordinary skill who wished to make and use the claimed invention. Rather, in practice, one of ordinary skill would not produce all the sequences of the claimed genus but would rather more likely produce a single sequence, which comprised a useful protein, falling within the scope of the claims. Thus they would start with, for example, a wild-type sequence, and make a small number of mutations, perhaps only one or two, in addition to the one or more specified

in the claim. They would then test to determine if these other mutations impacted adversely on the luciferase activity of the protein. If they did, then these mutation would be rejected and others tried. However, if luciferase activity were retained, then further mutations may be attempted on a similar basis. Such mutations are well within the scope of reasonable experimentation. The applicants should be entitled to protect all such sequences, and thus claiming homologues in terms of a percent identity is submitted to define the applicants contribution to the art.

The Examiner is again urged to appreciate that luciferases are highly conserved having a significant degree of similarity between them, as pointed out on page 7 lines 11 to 24 of the specification. Therefore it is reasonable to believe that a mutation, which is found to be effective in one luciferase species at improving one or more of its features, is going to be effective on other luciferases. The known structural similarity of luciferases is further supported by Ye et al.

It has been found, for example, that mutations of luciferases cause similar effects in different luciferase species. The following patent applications detail a number of other luciferase mutations, which have been found to have beneficial effects, WO95/18853 (a copy of which will be submitted separately), WO 96/22376 (a copy of which will be submitted separately) and WO 95/25798 (of record). Whilst the effect of mutating a luciferase at a new position could not be predicted in advance, it has been demonstrated that mutations that are found to be effective in a particular luciferase have the same or similar effect in other luciferases.

A copy of GB2301592 is attached to further illustrate this point. On page 1 of the specification the document states that " It is known that Japanese firefly luciferase

(*Luciola lateralis*) can be stabilised against heat inactivation by mutating at its position 217". The specification then goes on to demonstrate that the equivalent mutation in *Photinus pyralis* (215) also stabilises against heat inactivation. This can be seen in Figures 14 and 15 where it is shown that a 215 mutation causes an increase in heat stability over wild type luciferase. This example therefore demonstrates that mutations, which are found to be effective at improving the properties of one species of luciferase, can be predicted to have the same effect in other luciferase species.

The applicants have found that a mutation at position 214 is effective at increasing thermostability, in *Photinus pyralis*. It is submitted that predicting the effect of this new mutation was unpredictable before it had been found to be effective in *Photinus pyralis*. Because of the similarity between luciferases and the fact that in the past other mutations have been found to have the same effect in different luciferases, it is believed to be reasonably predictable that this mutation would be beneficial in another luciferase once it had been found to be beneficial in *Photinus pyralis*.

The Examiner is understood to assert that the specification is overly broad in scope because only a single working example of the invention is provided, and that this example allegedly fails to provide the necessary guidance for making or using the invention over the whole of the scope claimed. However, the applicants submit that the specification shows several examples of the claimed invention, for example, such as Examples 2 and 3 where the production of 2 mutants is described, Example 7 where 3 mutants are described and Example 8 where 3 further mutants are described. The specification is therefore submitted to provide sufficient direction to allow an ordinarily skilled person to make and use the claimed invention.

In particular, the specification states a number of possible amino acid substitutions. The specification also explains in detail on page 12 lines 34 to page 13 line 24, how proteins which fall within the scope of the claims can be identified by hybridisation and a method by which similarity/identity can be assessed.

The specification is submitted to teach one of ordinary skill how to make and use the claimed invention.

The Section 102 rejection of claims 1-2, 4-5, 7 and 21-24 over Embl Accession Number B25415, is moot in view of the above. The claims are submitted to be patentable over the published sequence and consideration of the following in this regard is requested.

The claims provide proteins which are believed to be patentably distinct from a wild-type sequence, such as the sequence of Accession Number D25415. The sequence Accession Number D25415 is believed to be the wild-type sequence PepJ19 described by Ye et al. The applicants believe this is clear from a comparison of the inventors' names on the Accession printout, namely Zeno S, Shiraishi S Inouye SnSaigo K, with the inventors shown on JP 9430398, which according to Ye et al (See, comments below Table 1 in Ye et al) as a source of the PepJ19 sequence. A printout of the bibliographic details of JP 9430398 and its U.S. equivalent are attached showing that the inventors are the same. The applicants believe therefore that the Accession No. D25415 describes, at best, a wild-type sequence which fails to anticipate the presently claimed recombinant sequence.

The Section 102 rejection of claims 1-2, 4-5, 7, 21-24 and 28-30 over Wood (U.S. Patent Application Publication 2003/006801) is moot in view of the above. The claims

are submitted to be patentable over Wood for the reasons similar to those mentioned above with regard to Accession Number D25415. Specifically, the sequence shown in Wood, which is believed to be relied upon by the Examiner, is also a wild-type sequence which fails to anticipate the present claims.

The Section 103 rejection of claim 25 over Gustafson (U.S. Patent No. 5,196,524) in view of Wood is moot in view of the above. The claims are submitted to be patentable over the combined teaching of Gustafson and Wood as the protein of claim 55, for example, which is similar to now canceled claim 25, is patentable and a plant cell transformed with the noted vector is also submitted to be patentable over the cited combination of art.

The claims are submitted to be patentable for the reasons noted above and a Notice to that effect is requested.

The obviousness-type double patenting rejection of claims 1-5, 7-8 and 21-23 over claims 1-4, 6-10, 14, 17-19 is "6-23" of copending pending application 10/111,723, is moot in view of the above. The Examiner is requested to hold in abeyance any further similar obviousness-type double patenting rejection until such time as allowable subject matter is identified, when the applicants will consider filing a Terminal Disclaimer to overcome the rejection.

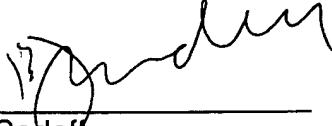
The Examiner is requested to contact the undersigned if anything further is required to advance the case to allowance.

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Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

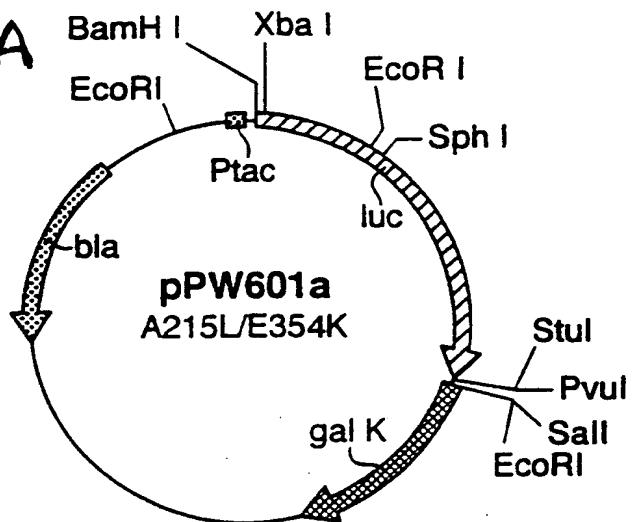

B. J. Sadoff
Reg. No. 36,663

BJS:

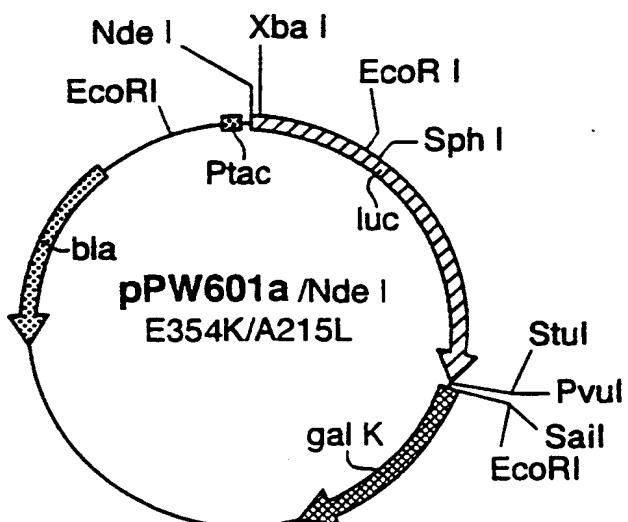
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Arlington, VA 22201-4714
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Facsimile: (703) 816-4100

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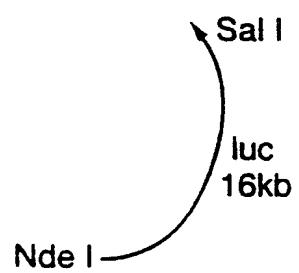
Fig. 1A



Site directed
mutagenesis
BamHI → Nde I

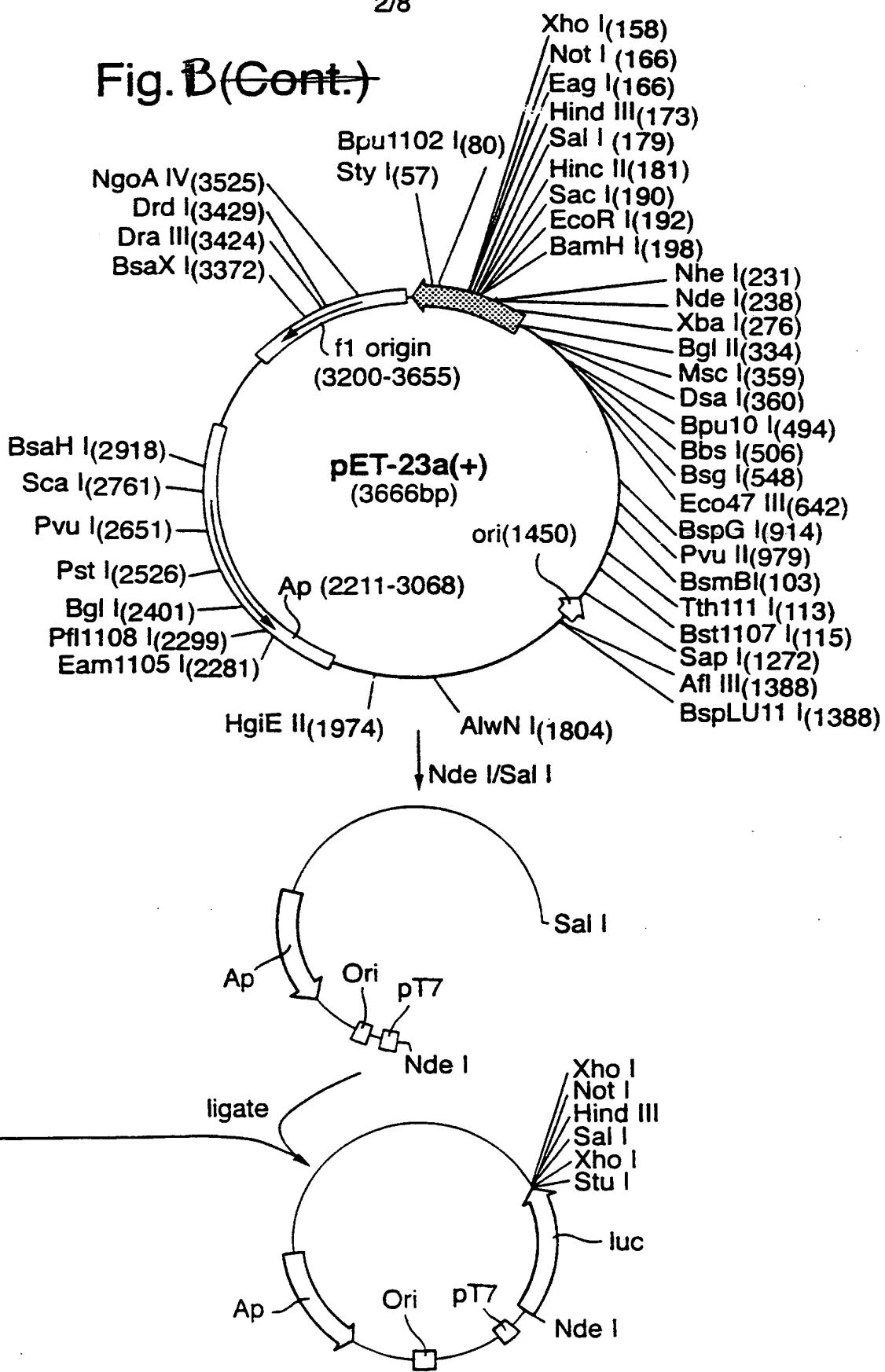


Nde I/Sal I



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Fig. B(Cont.)



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Fig.2.

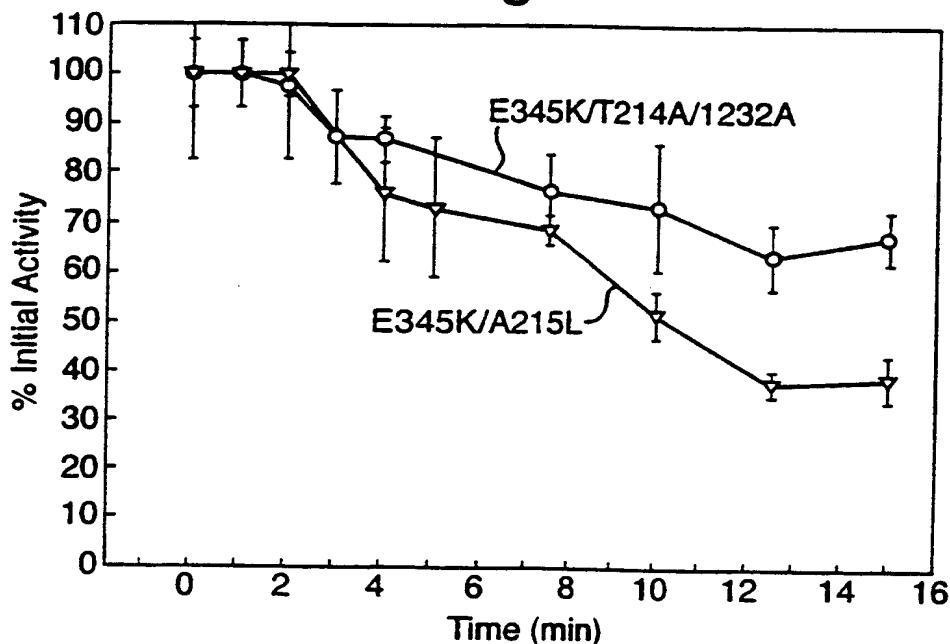
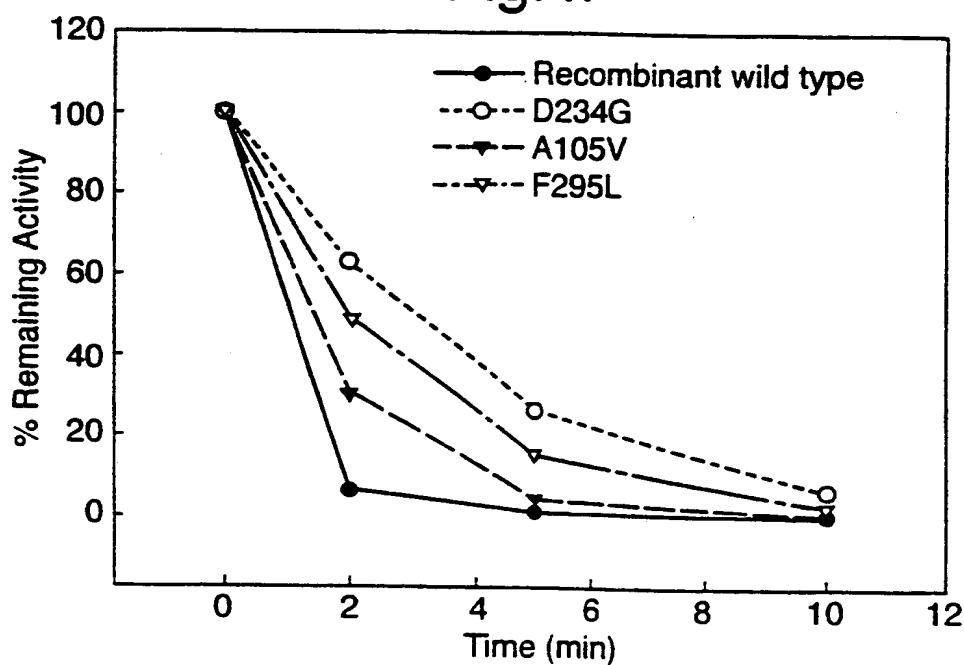


Fig.4.



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Fig. 3a. 3A

F295A

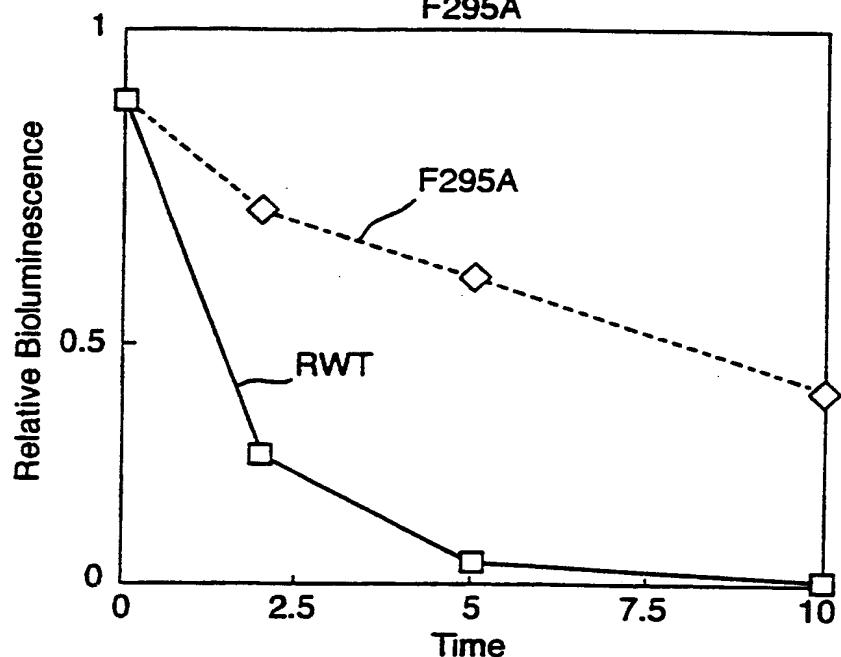
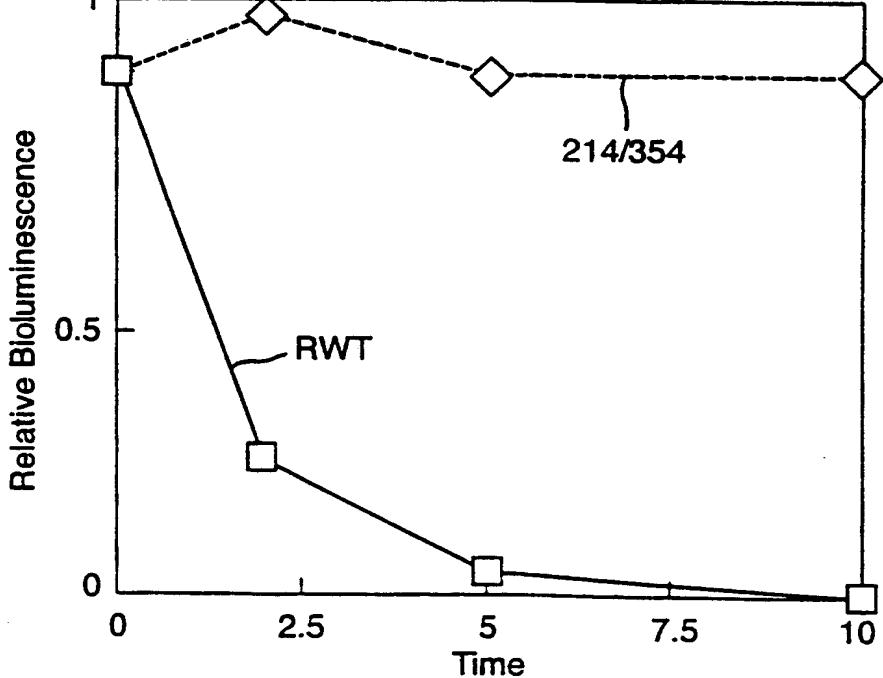


Fig. 3b. 3B

T214A & E354K mutations



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Fig.3e. 3C

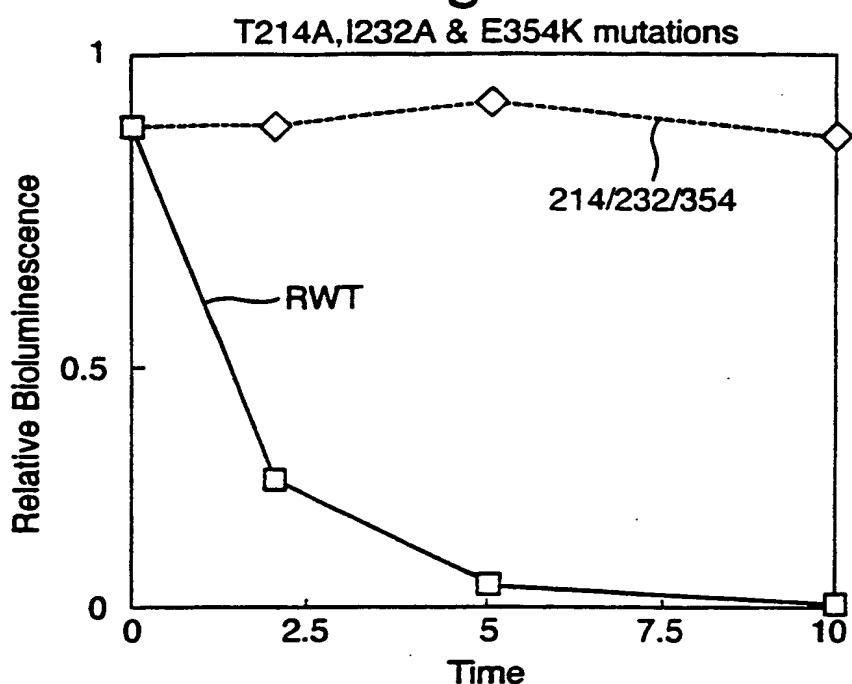
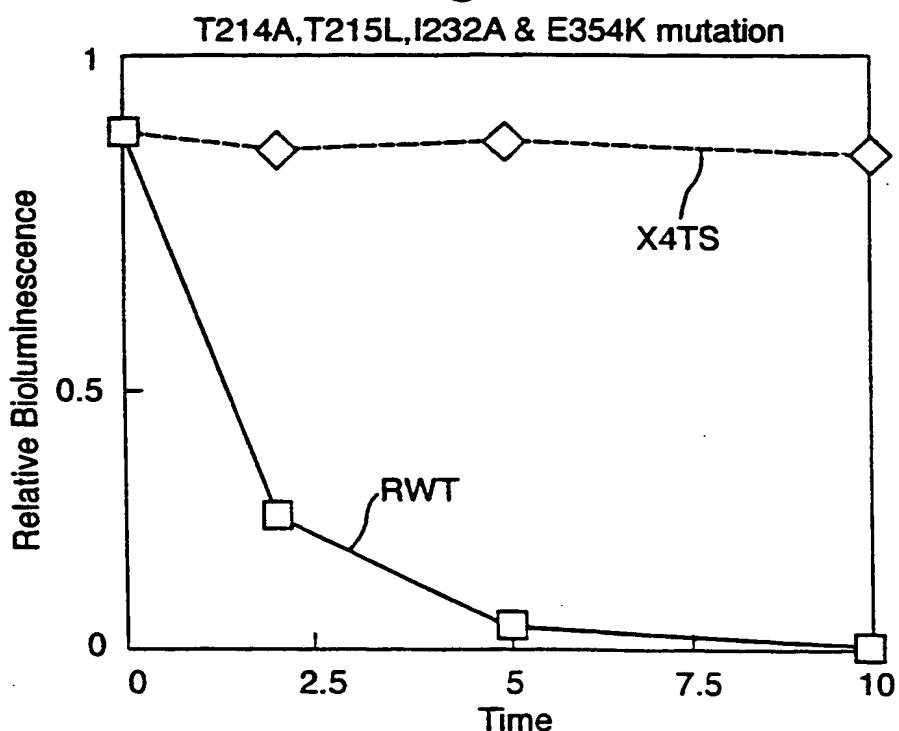


Fig.3d. 3D



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Fig.3e. 3E

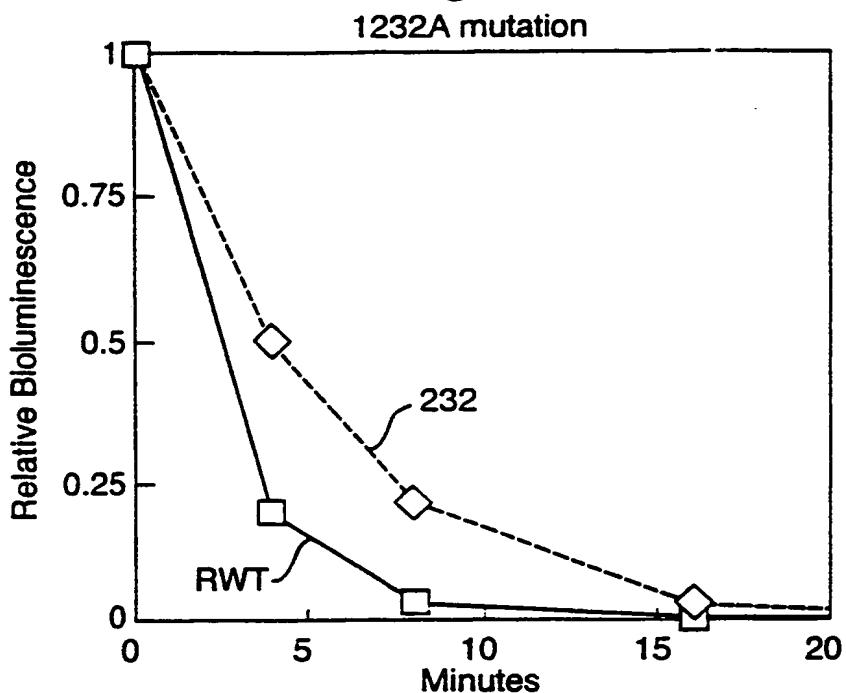


Fig.3f. 3F

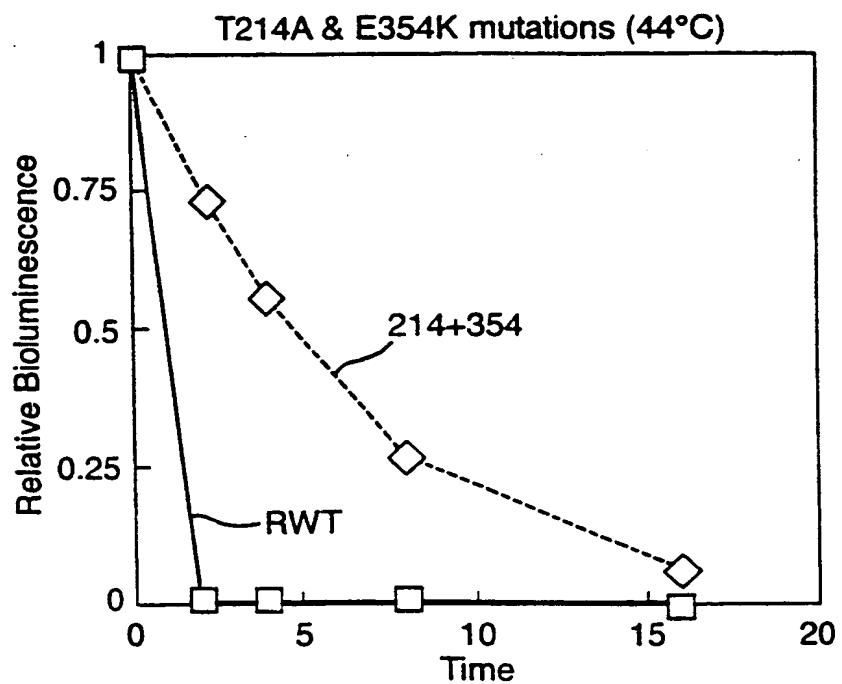


Fig.3g. 3G

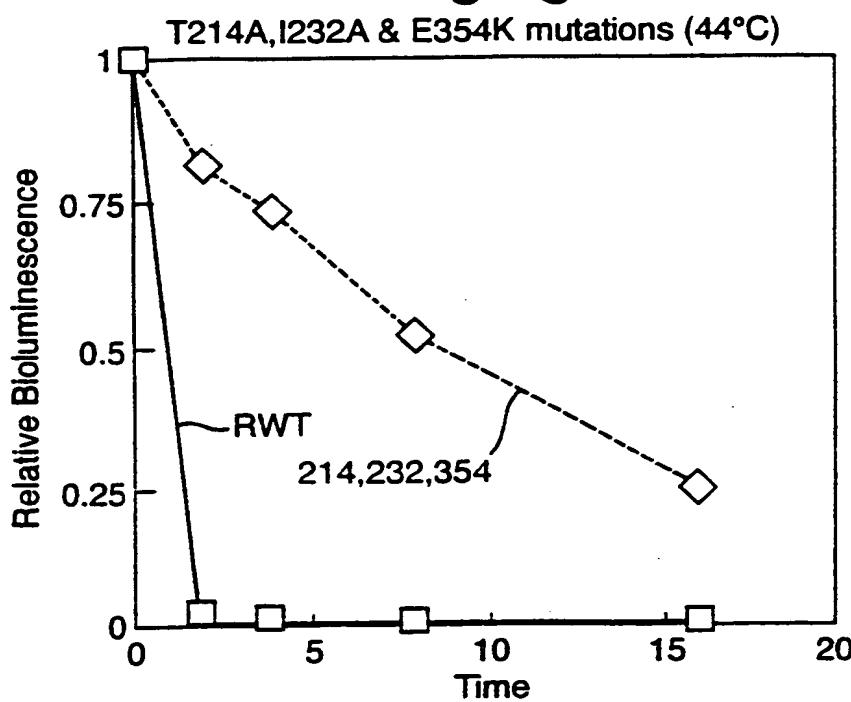
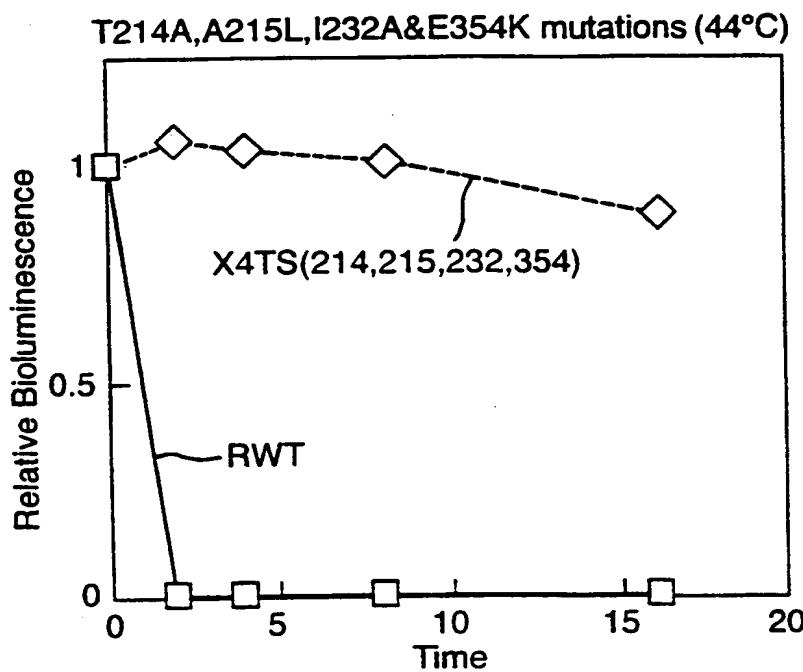


Fig.3h. 3H



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Fig. 5.

CGCCGGTGAGCTCCCCGCCGCCC	SACI-SENSE / 6371
CGGGCGGGGAGCTACCGGGC	SACI-ANTI / 6372
CGAACACTTCTTCATCGTTGACCCCTTAAGCTTTAATTAAATAACAAAGG	AFLII-SENSE / 6373
CCTTGTATTAAAGACTTAAGGCCTCAACTATGAAGAAGTGTTCG	AFLII-ANTI / 6374
GAAAGGCCGGCACAGCCTATCTTAGGG	F14A-SENSE / 6375
CCTCTAGGGATAGGCTGGTGCCCCCTTC	F14A-ANTI / 6376
CCATAAATTACCGAATTCGTCGACTTCGATCGAGG	C-TERMSEQ / 6341
GTGTGGAATTGTGAGCGG	N-TERMSEQ / 6651
GAGATACGCCCGGTTCTGG	L35A-SENSE / 6652
CCAGGAACCGCGGCGTATCTC	L35A-SENSE / 6653
CCCTATTTCATTCCTGGCCAAAAGCACTG	F295L-SENSE / 9048
GAGTGCTTTGGCCAGGAATGAAAATAGGG	F295L-ANTI / 9049
CCGCATAGAGCTCTCTGGTCAGATT	T214A + A215L-SENSE / 9063
GAATCTGACGCCAGAGAGCTCTATGCCG	T214A + A215L-ANTI / 9064
GTTGACCGTTGGATCCTTAATTAAATAC	Insertion of BamHI at G339 / 9077
GTATAGATTTGAAAAAGAGCTG	E270K-SENSE / 257
CAGCTCTTTCAAAATCTATAC	E270K-ANTI / 258
GGCTACATACTGGAGACATAGC	S420T-SENSE / 629
GCTATGTCCTCCAGTATGTAGCC	S420T-ANTI / 630
GCAGTTGGCCCGTGAACGAC	A105L-SENSE / 790
GTCGTTACGGCGCAACTGC	A105L-ANTI / 791
CAAATCATTGGGTACTGCATTTAAG	D234G-SENSE / 792
CTTAAATCGCAGTACCGGAATGATTG	D234G-ANTI / 793
CCGCATAGAACTCTCTGGTCAGATT	A215L-SENSE / 7728
GAATCTGACGCCAGAGAGCTCTATGCCG	A215L-ANTI / 7727
CTGATTACACCCAAGGGGATG	E354K-SENSE / 7792
CATCCCCCTGGGTGTAATCAG	E354K-ANTI / 7793
cccttcggatggnnnngctggcgt	T214N-Sense / 8202
actgacgcaggcNNNtcatggggaggc	T214N-Anti / 82033
GCAATCAAATCGCTCCGGATACTGC	I232A-SENSE / 6911
GCAGTATCCGGAGCGATTGATTGC	I232A-ANTI / 6912
CCATTCCATCAAGGTTTG	H245Q-SENSE / 9128
CCAAAACCTTGATGGAATGG	H245Q-ANTI / 9129